REMARKS/ARGUMENTS

Reconsideration and continued examination of the above-identified application are respectfully requested.

Rejection of Claims 1, 2, 7, 9 and 10 under 35 U.S.C. §103(a) over GB 1,005,024

At page 3 of the Office Action, the Examiner rejected claims 1, 2, 7, 9 and 10 under 35 U.S.C. §103(a) as being anticipated by GB 1,005,024. The Examiner alleged that GB 1,005,024 teaches compounds that read on the claimed formula of claim 1, except that R^3 of the formula depicted in claim 1 is limited to C_{1-3} alkyl, while in GB 1,005,024, the substituent at the corresponding position is hydrogen. The Examiner took the position that it would have been obvious to substitute methyl for hydrogen, on the alleged grounds that a person skilled in the art would have the expectation that structurally similar compounds would possess similar activity and pharmacological use. For the following reasons, this rejection is respectfully traversed.

It is respectfully submitted that a person skilled in the art would not have presumed that a compound according to GB 1,005,024 would possess similar activity to a compound of the claimed invention. In particular, persons skilled in the art of designing 5-HT receptor agonists and antagonists would not have been able to predict the effect of substituting a methyl group for a hydrogen in the naphthalene derivative of GB 1,005,024. As evidence to support this argument, the Applicants wish to direct the Examiner's attention to the publication, May et al. "Evaluation of the Ocular Hypotensive Response of Serotonin 5-HT_{1A} and 5-HT_2 Receptor Ligands in Conscious Ocular Hypertensive Cynomolgus Monkeys," *The Journal of Pharmacology and Experimental Therapeutics*, 2003, Vol. 306, No. 1, pp 301-309 ("May et al."), attached hereto as Exhibit 1. The May et al. article describes evaluations of 5-hydroxytryptamine ("5-HT") and

related derivatives, including 5-hydroxy-α-methyltryptamine ("5-HOAMT") in terms of 5-HT_{1A} and 5-HT_2 receptor binding, 5-HT_{1A} and 5-HT_2 receptor activity and efficacy in reducing intraocular pressure in test animals. The Examiner should note that the relationship between 5-HT and 5-HOAMT is analogous to the relationship between the compound of GB 1,005,024 and the compound of the present invention wherein R^3 is CH_3. In particular, in both sets of compounds, there is a core structure with an attached aminoethyl group in the first compound of the set and an aminopropyl group in the second compound of the set. In the case of 5-HT and 5-HOAMT, it is reported at page 304, Table 2, of May et al. that these two compounds have roughly similar receptor binding affinities of the 5-HT_2 receptors. Nevertheless, the *in vivo* data demonstrates that there is an unexpected and significant difference between the compounds in their activity with respect to the lowering of intraocular pressure ("IOP response"). As shown in Figure 1A of May et al., the IOP response of 5-HOAMT is far greater than that of 5-HT in the third and fifth hours post dose. The relevant data of May et al. regarding this point is summarized in table form in "Physicochemical Data and Monkey IOP Response Data," attached hereto as Exhibit 2.

Therefore, contrary to what is alleged by the Examiner, a person skilled in the art would not have an expectation that the compound of GB 1,005,024 and the compound of the present invention wherein R^3 is CH_3 would possess similar *in vivo* activity and therefore could not derive motivation from structural similarity to make the claimed compounds. Accordingly, the Examiner has not established a *prima facie* case of obviousness. Therefore, the rejection should be withdrawn.

Regarding claims 9 and 10, these claims are directed to a composition comprising a compound of Formula I and at least one ophthalmologically acceptable carrier. Because GB

1,005,024 does not teach or suggest an ophthalmic composition comprising its compound, claims 9 and 10 are clearly allowable over GB 1,005,024 for this additional reason.

Moreover, Applicants specifically request that upon allowance of the compound claims, the Examiner rejoin and allow all of the method claims, including claims 3 - 6, 8, and 11 - 14.

CONCLUSION

In view of the foregoing remarks, the applicant respectfully requests the reconsideration of this application and the timely allowance of the pending claims.

If there are any other fees due in connection with the filing of this response, please charge the fees to Deposit Account No. 50-0925. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such extension is requested and should also be charged to said Deposit Account.

Respectfully submitted,

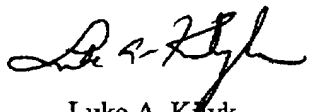

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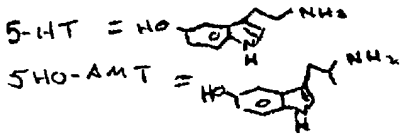
EXHIBIT 1



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Evaluation of the Ocular Hypotensive Response of Serotonin 5-HT_{1A} and 5-HT_2 Receptor Ligands in Conscious Ocular Hypertensive Cynomolgus Monkeys

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ABSTRACT

Published investigations of serotonin-1A (5-hydroxytryptamine$_{1A}$; 5-HT_{1A}) receptor agonists and serotonin-2A (5-hydroxytryptamine$_{2A}$; 5-HT_{2A}) receptor antagonists in nonprimate species provide conflicting results with regard to their intraocular pressure-lowering efficacy. Thus, their therapeutic utility in the treatment of human glaucoma has been confusing. We evaluated the effect of selected 5-HT_{1A} agonists and 5-HT_{2A} receptor antagonists on intraocular pressure in a nonhuman primate model, the conscious cynomolgus monkey with laser-induced ocular hypertension. Neither selective 5-HT_{1A} agonists [e.g., *R*-8-hydroxy-2-(di-*n*-propylamino)tetralin and flesinoxan] nor selective 5-HT_2 receptor antagonists [e.g., *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol (M-100907) and 6-chloro-2,3-dihydro-5-methyl-*N*-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1*H*-indole-1-carboxamide (SB-242084)] lowered intraocular pressure in the primate model following topical ocular administration. However, compounds that function as agonists at both the 5-HT_{1A} and 5-HT_2 receptors were found to effectively lower intraocular pressure in the model: 5-hydroxy-α-methyltryptamine, 5-methoxy-α-methyltryptamine, 5-hydroxy-*N*,*N*-dimethyltryptamine (bufotenine), and 5-methoxy-*N*,*N*-dimethyltryptamine. Furthermore, the selective 5-HT_2 receptor agonist *R*-(−)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane lowered intraocular pressure in the primate model, demonstrating a pharmacological response associated with activation of the 5-HT_2 receptor. These observations suggest that compounds that function as efficient agonists at 5-HT_2 receptors should be considered as potential agents for the control of intraocular pressure in the treatment of ocular hypertension and glaucoma in humans.

Identification of several of the numerous serotonin receptors in ocular tissues of the anterior segment of the eye, including the iris-ciliary body of rabbit (Chidlow et al., 1998) and human (Martin et al., 1992), suggests that this neurotransmitter may play an important role in the regulation of intraocular pressure (IOP). Serotonin (5-hydroxytryptamine; 5-HT) is also found in the aqueous humor of humans (Veglio et al., 1998) and other mammals (Boerrigter et al., 1992). These observations have generated considerable interest in the role that serotonin might have in aqueous humor dynamics and even whether 5-HT might be involved in the development of ocular hypertension and glaucoma. There have been numerous conflicting reports on the effect of 5-HT and various 5-HT receptor ligands on IOP in the rabbit, dog, and humans. These observed differences in IOP response may be due to species differences, route of administration, or the lack of 5-HT receptor selectivity of the agents evaluated. For example, 5-HT has been shown to lower IOP in rabbits following intravenous injection (Chiang, 1974). However, when injected intracamerally in the rabbit, a rise in IOP was observed (Krootila et al., 1987). Furthermore, topical ocular administration of 5-HT to the rabbit was reported to result in either a decrease (Krootila et al., 1987) or an increase (Meyer-Bothling et al., 1993) in IOP.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
DOI: 10.1124/jpet.103.049528.

ABBREVIATIONS: IOP, intraocular pressure; 5-HT, 5-hydroxytryptamine (serotonin); 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; 5-CT, 5-carboxamidotryptamine; SB-206553, 5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole; M-100907, *R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol; SB-242084, 6-chloro-2,3-dihydro-5-methyl-*N*-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1*H*-indole-1-carboxamide; DOI, 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane; *R*-DOI, *R*-(−)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane; CHO, Chinese hamster ovary; MAO, monoamine oxidase; DMEM, Dulbecco's modified Eagle's medium; FLIPR, fluorescence imaging plate reader; DMSO, dimethyl sulfoxide; 5-MeODMT, 5-methoxy-*N*,*N*-dimethyltryptamine; 5-HOAMT, 5-hydroxy-α-methyltryptamine; 5-MeOAMT, 5-methoxy-α-methyltryptamine; DP-5-CT, *N*,*N*-dipropyl-5-carboxamidotryptamine; 5-MeOT, 5-methoxytryptamine; 5-HOMT, 5-hydroxy-*N*-methyltryptamine; 5-HODMT, 5-hydroxy-*N*,*N*-dimethyltryptamine (bufotenine); RS 102221, 8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione hydrochloride; WB-4101 [2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride.

The 5-HT_{1A} receptor agonist *R*-(+)-8-hydroxy-2-(di-*n*-propylamino)tetralin (*R*-8-OH-DPAT) has been reported to decrease IOP in normal rabbits by activating 5-HT_{1A} receptors in the ciliary body and α_2-adrenoceptors in the brain (Chu et al., 1999). Another study reports that the decrease in IOP following topical administration of *R*-8-OH-DPAT to normotensive rabbits is due to a local stimulation of 5-HT_{1A} receptors in the anterior uvea (Osborne et al., 2000). Also, flesinoxan, a selective potent full agonist at the 5-HT_{1A} receptor, has been reported to lower IOP in normotensive rabbits following topical administration, but this response may be attributable to the α_1-antagonist activity of flesinoxan (Chidlow et al., 2001). The nonselective 5-HT_{1A} agonist 5-CT [5-carboxamidotryptamine] has been reported to increase IOP following topical ocular application to rabbits (Meyer-Bothling et al., 1993). Further evaluation of this compound suggested that the ocular hypertensive response to 5-CT observed in the rabbit was caused by activation of an irritative pathway unrelated to agonist activity at the 5-HT_{1A} receptor (Chidlow et al., 1999).

Topical ocular administration of 5-HT_2 receptor antagonists resulted in a decrease in IOP in monkeys (Chang et al., 1985) and humans (Mastropasqua et al., 1997), suggesting utility for such compounds in the treatment of ocular hypertension associated with glaucoma. The 5-HT_2 receptor antagonists ketanserin (Mastropasqua et al., 1997) and sarpogrelate (Takenaka et al., 1995) have been shown to significantly lower IOP in glaucoma patients. However, both of these compounds also have potent antagonist activity at the α_{1A} receptor. The ocular hypotensive response observed with these agents is likely mediated through their α_1-adrenergic antagonist activity and not their 5-HT_2 antagonist activity.

The purpose of the present study was to determine the ocular hypotensive activity of 5-HT_{1A} receptor agonists and 5-HT_2 receptor antagonists in our conscious cynomolgus monkey model of laser-induced ocular hypertension. This is a model that we have found to be highly predictive of ocular hypotensive activity in humans (Hellberg et al., 2001; Sharif et al., 2001). Toward this end, we chose to evaluate both selective and nonselective 5-HT_{1A} agonists and selective 5-HT_2 antagonists. In view of the lack of specificity of the agents used to characterize the ocular hypotensive activity and the conflicting results noted in previous in vivo studies (vide supra), it was important to obtain a detailed in vitro profile of the 5-HT_{1A} agonists to be evaluated in the in vivo model. We report here that neither 5-HT_{1A} agonists nor 5-HT_2 antagonists decreased IOP in the monkey. However, the in vitro functional response profile of the nonselective 5-HT_{1A} agonists that were evaluated led us to identify potent 5-HT_2 receptor agonists as effective agents for lowering IOP in the monkey.

Materials and Methods

Chemicals

Serotonin hydrochloride, *R*-(+)-8-hydroxy-2-(di-*n*-propylamino)-tetralin hydrobromide, *N,N*-dipropyl-5-carboxamidotryptamine maleate, 5-methyl-urapidil, α-methyl-5-hydroxytryptamine maleate, 5-methoxytryptamine hydrochloride, ketanserin, cinanserin, ritanserin, SB-206553, (±)-, *S*-(+)-, and *R*-(−)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride, *N,N*-dimethyl-5-methoxytryptamine, *N*-methyltryptamine oxylate, and 5-fluoro-α-methyl-tryptamine were purchased from Sigma/RBI (Natick, MA). RS-102221 hydrochloride and WB-4101 hydrochloride were obtained from Tocris Cookson Inc. (Ballwin, MO). Flesinoxan hydrochloride was obtained from Solvay Pharma BV (Weesp, The Netherlands). α-Methyl-5-methoxytryptamine hydrochloride was obtained from the National Institute of Mental Health's Chemical Synthesis and Drug Supply Program (SRI International, Menlo Park, CA). Bufotenine (*N,N*-dimethyl-5-hydroxytryptamine) oxalate was obtained from Biosynth International (Naperville, IL). Oxylate salts were converted to the fumarate salts, which were used for the in vivo studies. M-100907 (*R*-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol) and SB-242084 (6-chloro-2,3-dihydro-5-methyl-*N*-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-1*H*-indole-1-carboxamide) were synthesized by the reported procedures (Bromidge et al., 1997; Ullrich and Rice, 2000). Radioligands [^{125}I](±)-DOI (2200 Ci/mmol), [^{3}H]8-hydroxy-DPAT (135 Ci/mmol), and [^{3}H]clonidine (55.5 Ci/mmol) were obtained from PerkinElmer Life Sciences (Boston, MA). Frozen adult rat brains, rapidly harvested and frozen at −20°C after sacrifice, were obtained from Pelfreeze (Brown Deer, WI).

Cell Lines

Chinese hamster ovary (CHO) cell membranes expressing the recombinant human 5-HT_{1A} receptor were obtained from PerkinElmer Life Sciences, and CHO cells expressing the cloned human 5-HT_{1A} receptor were obtained from Euroscreen (Brussels, Belgium). Membranes from Sf9 cells expressing the cloned human α_{2A}- and α_{2C}-adrenergic receptor were obtained from BioSignal Packard, Inc. (Montreal, PQ, Canada).

In Vitro Binding Assays

Determination of Binding to Cloned Human 5-HT_{1A} Receptors. The binding of [^{3}H]8-OH-DPAT (0.25 nM final) to CHO cell membranes expressing the recombinant human 5HT_{1A} receptor was performed in 50 mM Tris-HCl buffer (pH 7.4) in a total volume of 0.5 ml for 1 h at 27°C. The test compounds, along with the positive control compound [(*R*)-8-OH-DPAT], were tested over 5- to 10-log unit concentrations for their ability to compete for [^{3}H]8-OH-DPAT binding. The assays were terminated by rapid vacuum filtration over glass fiber filters previously soaked in 0.3% polyethyleneimine. The radioactivity was counted on a β-counter, and the data were analyzed by a nonlinear, iterative, curve-fitting computer program.

Determination of Binding to 5-HT_{2A} Receptor. To determine the relative affinities of serotonergic compounds at the 5-HT_2 receptors, their ability to compete for the binding of the agonist radioligand [^{125}I](±)-DOI to brain 5-HT_{2A} receptors was determined as described here with minor modification of the literature procedure (Johnson et al., 1987). Aliquots of postmortem rat cerebral cortex homogenates (400 μl) dispersed in 50 mM Tris-HCl buffer (pH 7.4) were incubated with [^{125}I](±)-DOI (80 pM final) in the absence or presence of methiothepin (10 μM final) to define total and nonspecific binding, respectively, in a total volume of 0.5 ml. The assay mixture was incubated for 1 h at 25°C in polypropylene tubes, and the assays were terminated by rapid vacuum filtration over Whatman GF/B glass fiber filters previously soaked in 0.3% polyethyleneimine using ice-cold buffer. Nonspecific binding was defined with 1 to 10 μM methiothepin. Filter-bound radioactivity was determined by liquid scintillation spectrometry on a beta counter. The data were analyzed using a nonlinear, iterative curve-fitting computer program (Bowen and Jerman, 1995) to determine the compound affinity parameter. The concentration of the compound needed to inhibit the [^{125}I](±)-DOI binding by 50% of the maximum (IC_{50} value) was determined for each compound.

Determination of Binding at Cloned Human 5-HT_2 Receptors. Binding affinity of compounds at the cloned human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors expressed in Chinese hamster ovary

cells using the agonist [^{125}I](±)-DOI (0.2 nM; 15 min at 37°C) as the radioligand for each receptor was determined and reported as K_i values. These studies were conducted at Cerep, Poitiers, France using radioligand binding techniques similar to those described above.

Determination of Binding at Cloned Human α_{2A} and α_{2C} Receptors. Membranes from Sf9 cells expressing the cloned human α_{2A} and α_{2C} receptors were diluted to 32 μg/ml and 48 μg/ml protein, respectively, in 75 mM Tris-HCl containing 12.5 mM $MgCl_2$ and 2 mM EDTA (pH 7.4). The membranes were resuspended using a Branson Sonifier 450 (Branson Ultrasonics Corp., Danbury, CT) (<20 s). Drug dilutions were made in 1:1:8 DMSO/ethanol/water (v/v) using a Biomek 2000 robot (Beckman Coulter, Inc., Fullerton, CA). The diluted compounds (25 μl), followed by a volume of 200 μl of receptor preparation and, finally, 25 μl of [^{3}H]clonidine (28 nM final concentration) were added by the Biomek 2000 robot to a 96-well plate. The incubations (60 min at 23°C) were terminated by rapid vacuum filtration on a TomTec Harvester 96 (TomTec, Orange, CT) using Whatman GF/C glass fiber filters that were previously soaked in 0.3% polyethyleneimine. The filters were washed with ice-cold 50 mM Tris-HCl, pH 7.4. The samples were counted on a TopCount scintillation counter (PerkinElmer Life Sciences).

Determination of Other Receptor Binding Activity. Binding assays for 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_3$, 5-HT$_4$, 5-HT$_6$, and 5-HT$_7$ serotonergic receptors and α_{1A}-, α_{1B}-, and α_{2B}-adrenergic receptors were conducted at NovaScreen Biosciences (Hanover, MD), using their standard screening protocols.

Determination of Monoamine Oxidase Activity. *Monoamine oxidase A.* Aliquots of rat brain homogenate were preincubated with Ro 41–1049, test compound, and 100 nM deprenyl for 60 min at 37°C in 50 mM K_2HPO_4 containing 50 μM EDTA and 10 μM dithiothreitol (pH 7.2 at 25°C). Substrate ([^{14}C]serotonin, 50 μM) was then added and incubated for 30 min. The reaction was stopped by the addition of 0.5 ml of 1 to 2 M citric acid. Radioactive product was extracted into a xylene/ethyl acetate fluor and compared with control values by scintillation spectrophotometry to ascertain any interactions of test compounds with MAO-A.

Monoamine oxidase B. Aliquots of rat brain homogenate were preincubated with Ro 16–6491, test compound, and 100 nM clorgyline for 60 min at 37°C in 50 mM K_2HPO_4 containing 50 μM EDTA and 10 μM dithiothreitol (pH 7.2 at 25°C). Substrate ([^{14}C]phenylethylamine, 10 μM) was then added and incubated for 10 min. The reaction was stopped by the addition of 0.5 ml of 1 to 2 M citric acid. Radioactive product was extracted into a xylene/ethyl acetate fluor and compared with control values by scintillation spectrophotometry to ascertain any interactions of test compounds with MAO-B.

In Vitro Functional Assays

Determination of 5-HT$_{1A}$ Activity: cAMP Production in Cultured Cells. CHO cells expressing the cloned human 5-HT$_{1A}$ receptor were maintained in CHO-S-SFMII medium containing 0.4 mg/ml G418, 2.5 μg/ml fungizone, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1.0% dialyzed fetal bovine serum. The cells were cultured in 48-well plates, maintained in a humidified atmosphere of 5% CO_2 and 95% air, and fed twice weekly. Upon reaching confluence, the cells were rinsed twice with 0.5 ml of DMEM/F-12. The sample wells were then preincubated for 20 min with DMEM/F-12 containing 0.8 mM ascorbate and a 1.0 mM concentration of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine, at 23°C. The test compounds (6-log unit concentrations) were then added to the cells for 20 min, followed by the addition of forskolin (10 μM), and the incubation continued for another 10 min at 23°C. After aspiration of the reaction medium, ice-cold 0.1 M acetic acid (150 μl, pH 3.5) was added for the termination of cAMP synthesis and cell lysis. Finally, ice-cold 0.1 M sodium acetate (225 μl, pH 11.5–12.0) was added to neutralize the samples. The measurement of cAMP was performed using an enzyme immunoassay. The assay was conducted according to the package insert for the enzyme immunoassay kit in an automated manner using the Biomek 2000 robot system.

Determination of 5-HT$_2$ Activity: [Ca^{2+}]$_i$ Mobilization Assay. The receptor-mediated mobilization of intracellular calcium ([Ca^{2+}]$_i$) was studied using the Fluorescence Imaging Plate Reader (FLIPR) instrument. Rat vascular smooth muscle cells, A7r5, were grown in a normal medium of DMEM/10% fetal bovine serum and 10 μg/ml gentamicin. Confluent cell monolayers were trypsinized, pelleted, and resuspended in normal medium. Cells were seeded in a 50-μl volume at a density of 20,000 cells per well in a black-wall, 96-well tissue culture plate and grown for 2 days. On the day of the experiment, one vial of FLIPR Calcium Assay Kit dye was resuspended in 50 ml of a FLIPR buffer consisting of Hanks' balanced salt solution, 20 mM HEPES, and 2.5 mM probenecid, pH 7.4. Cells were loaded with the calcium-sensitive dye by addition of an equal volume (50 μl) to each well of the 96-well plate and incubated with dye for 1 h at 23°C. Typically, test compounds were stored at 25 μM in 50% DMSO/50% ethanol solvent. Compounds were diluted 1:50 in 20% DMSO/20% ethanol. For dose-response experiments, compounds were diluted 1:50 in FLIPR buffer and serially diluted 1:10 to give a five- or eight-point dose-response curve.

At the beginning of an experimental run, a signal test was performed to check the basal fluorescence signal from the dye-loaded cells and the uniformity of the signal across the plate. The basal fluorescence was adjusted between 8,000 and 12,000 counts by modifying the exposure time, the camera F-stop, or the laser power. The instrument settings for a typical assay were as follows: laser power 0.3 to 0.6 W, camera F-stop F/2, and exposure time 0.4 s. An aliquot (25 μl) of the test compound was added to the existing 100-μl dye-loaded cells at a dispensing speed of 50 μl/s. Fluorescence data were collected in real-time at 1.0-s intervals for the first 60 s and at 6.0-s intervals for an additional 120 s. Responses were measured as peak fluorescence intensity minus basal and, where appropriate, were expressed as a percentage of a maximum 5-HT-induced response.

Animal Studies

Acute IOP Response in Conscious Cynomolgus Monkeys. IOP was determined with an Alcon Pneumatonometer after light corneal anesthesia with 0.1% proparacaine. Eyes were rinsed with saline after each measurement. After a baseline IOP measurement, test compound was instilled in one 30-μl aliquot to the test eyes (either ocular hypertensive or normotensive) of eight to nine cynomolgus monkeys. Vehicle was instilled in the test eyes of five to six additional animals. Subsequent IOP measurements were taken at 1, 3, and 6 h. A compound is considered efficacious in hypertensive eyes if there is a decrease from baseline IOP of at least 20% following topical administration.

Formulation. Compounds were formulated in phosphate-buffered saline vehicle containing 0.01% benzalkonium chloride, 0.01% disodium EDTA, 0.1% polysorbate 80, 0.8% hydroxypropylmethylcellulose and adjusted to pH 7.4

Statistical Analysis. An SAS computer program (Job PC235; SAS Institute, Cary, NC) performed Student's *t* test to compare differences in IOP from baseline for each time point and one-way analysis of variance to compare differences in IOP between groups for each time point.

Animal Management. All nonhuman primates were cynomolgus monkeys (*Macaca fascicularis*) received from Charles River Laboratories or Covance Research Products (Denver, PA). Animals were male and female adults that were part of a permanent colony. Each animal was permanently identified with a unique number tattoo on the abdomen. Previously, hypertension had been induced in the right eyes of all animals by laser trabeculoplasty. All left eyes were normal and normotensive. Animals had been trained to sit in restraint chairs and conditioned to accept the pressure measurements without chemical restraint. The animals were housed singly in stainless steel squeeze-back suspended wire-bottom cages, had access to tap water ad libitum, and were fed a standard certified laboratory primate diet

TABLE 1

IC_{50} values, agonist potencies (EC_{50}), and relative intrinsic activities (RIAs) of compounds at the cloned human 5-HT_{1A} receptor and the rat 5-HT_{2A} receptor

Values are the mean of at least three experiments ± S.E.M.

Compound	5-HT_{1A}		5-HT_{2A}	
	IC_{50}[a]	EC_{50}[b] (RIA)[c]	IC_{50}[d]	EC_{50}[e] (RIA)[f]
	nM		*nM*	
5-HT	0.87 ± 0.26	24.9 ± 9.53 (93%)	0.94 ± 0.07	57.9 ± 4.84 (99%)
(*R*)-8-OH-DPAT	0.52 ± 0.03	2.59 ± 0.52 (102%)	336 ± 36	>10,000
DP-5-CT	0.19 ± 0.03	0.25 ± 0.06 (97%)	1,148 ± 398	>10,000
Flesinoxan	0.20 ± 0.08	7.83 ± 0.93 (96%)	4,525 ± 1,294	N.D.
5-Me-urapidil	0.13 ± 0.06	0.71 ± 0.16 (84%)	11,200 ± 793	>10,000
WB-4101	1.34 ± 0.21	7.34 ± 2.32 (77%)	N.D.	N.D.
5-MeOT	1.47 ± 0.67	9.85 ± 0.52 (105%)	5.74 ± 3.00	43.3 ± 9.76 (94%)
5-HOMT	1.21 ± 0.14	1.53 ± 0.42 (104%)	4.51 ± 0.49	76.6 ± 4.93 (63%)
5-HODMT	5.56 ± 2.42	39.6 ± 5.65 (96%)	1.76 ± 0.05	67.5 ± 2.50 (43%)
5-MeODMT	4.52 ± 1.50	64 ± 26 (104%)	9.53 ± 3.67	462 ± 70.4 (34%)
5-HOAMT	19.6 ± 6.17	378 ± 140 (111%)	1.54 ± 0.24	51.5 ± 3.24 (99%)
5-MeOAMT	86.4 ± 18.7	564 ± 181 (92%)	2.65 ± 1.31	47.5 ± 1.94 (96%)
rac-DOI	4,230 ± 740	N.D.	0.33 ± 0.04	30.2 ± 10.7 (31%)
R-DOI	3,843 ± 726	>10,000	0.21 ± 0.06	17.8 ± 4.16 (33%)
S-DOI	4,050 ± 167	N.D.	1.31 ± 0.57	47.4 ± 3.22 (26%)

N.D., not determined.
[a] Radioligand [^{3}H]8-OH-DPAT, CHO cell membranes expressing the recombinant human 5HT_{1A} receptor.
[b] CHO cells expressing the cloned human 5HT_{1A} receptor.
[c] Values in parentheses, relative to maximum 5-HT-induced response.
[d] Radioligand [^{125}I]DOI, homogenized rat cerebral cortex.
[e] Intracellular calcium mobilization in rat vascular smooth muscle cells (A7r5).

[PMI (Purina Mills, Inc.) Certified Primate Diet] twice daily and supplemental fresh fruit. No contaminants were known to be present in the diet or drinking water that would interfere with or affect the ocular studies. Lighting in the animal room was controlled to give 14 h light and 10 h dark each day. Room temperature was maintained at an average 25°C. Humidity was maintained at ≥35%. Animals were transferred from holding cages to restraint chairs using the pole-and-collar method, a procedure to which all animals had been trained. Animals were in the chairs for no longer than 8 h at a time. Animal studies were conducted in accordance with the resolutions for the use of laboratory animals as adopted by the National Institutes of Health and the Association for Research in Vision and Ophthalmology.

Results

In Vitro Assays

Profile of Ligands at Serotonergic Receptors. A comparative binding profile of the compounds of the present study at the cloned human 5-HT_{1A} receptor and at the 5-HT_{2A} receptor isolated from rat cortex is summarized in Table 1. The functional response of the compounds at these receptors is also presented. The selective 5-HT_{1A} receptor agonists investigated (e.g., *R*-8-OH-DPAT, flesinoxan, 5-Me-urapidil) showed greater than 600-fold higher affinity for the cloned 5-HT_{1A} receptor than for the rat 5-HT_{2A} receptor, and the various methylated analogs of 5-HT of interest were agonists at both the 5-HT_{1A} and 5-HT_{2A} receptors (Table 1). The activity of the latter compounds ranged from potent full agonist activity at 5-HT_{1A} and moderately potent partial agonist activity at 5-HT_{2A} (5-methoxy-*N*,*N*-dimethyltryptamine, 5-MeODMT) to potent full agonist activity at 5-HT_{2A} and moderately potent full agonist activity at 5-HT_{1A} (5-hydroxy-α-methyltryptamine, 5-HOAMT) (Table 1). The selective 5-HT_2 receptor agonist *R*-DOI showed low affinity for, and no functional activity at, the 5-HT_{1A} receptor (Table 1).

Those compounds that displayed agonist activity at the rat brain 5-HT_{2A} receptor were evaluated for their affinity at the cloned human 5-HT_2 receptor subtypes (Table 2). In general, none of the substituted 5-HT derivatives of this study showed a profound selectivity for any of the 5-HT_2 receptor subtypes. Of the tryptamines evaluated, 5-MeODMT showed approximately a 3-fold selectivity for 5-HT_{2A} versus 5-HT_{2B} or 5-HT_{2C}, whereas 5-HOAMT had approximately a 2-fold higher affinity for 5-HT_{2C} than for 5-HT_{2A} or 5-HT_{2B}. A modestly increased selectivity was observed for the phenylethylamine ligands (±)-DOI and *R*-DOI, which have an approximately 7-fold higher affinity for 5-HT_{2A} than for 5-HT_{2C}, and a 12- and 28-fold selectivity for 5-HT_{2A} versus 5-HT_{2B}, respectively (Table 2).

The binding affinity at other 5-HT receptors was determined for four of the compounds of particular interest: 5-MeODMT, 5-HOAMT, 5-methoxy-α-methyltryptamine (5-MeOAMT), and *R*-DOI (Table 3). In general, these compounds showed low to moderate affinity at other 5-HT receptors. However, 5-MeODMT and 5-MeOAMT showed high affinity at the 5-HT_7 receptor, and 5-MeODMT and 5-HOAMT showed good affinity at the 5-HT_{1D} receptor.

TABLE 2

Binding affinities of compounds at cloned human 5-HT_2 receptor subtypes expressed in CHO cells

Values are the mean of three experiments ± S.E.M.

Compound	K_i for [^{125}I]DOI Binding		
	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
	nM		
5-HT	8.2 ± 1.6	13 ± 4.5	8.3 ± 2.6
5-MeOT	4.7 ± 0.20	11 ± 3.9	7.5 ± 1.7
5-HOMT	15 ± 1.5	22 ± 5.0	23 ± 5.2
5-HODMT	10 ± 0.52	14 ± 2.9	23 ± 7.0
5-MeODMT	15 ± 2.3	52 ± 2.0	42 ± 7.5
5-HOAMT	12 ± 2.2	13 ± 3.2	6.6 ± 0.60
5-MeOAMT	4.6 ± 0.79	7.8 ± 1.4	8.3 ± 2.2
rac-DOI	0.96 ± 0.15	12 ± 1.0	7.2 ± 2.0
(*R*)-DOI	0.65 ± 0.07	18 ± 2.7	4.0 ± 0.80
(*S*)-DOI	3.3 ± 1.1	21 ± 5.2	10 ± 2.6

TABLE 3

Binding affinities (K_i; micromolar) for selected compounds at other 5-HT receptors and the uptake site

Determinations were done by NovaScreen Biosciences, using their standard protocols. Inhibition constants (K_i) were determined using up to seven concentrations of each compound. Each value on the concentration plot was the mean of two determinations.

Compound	5HT$_{1B}$[a]	5HT$_{1D}$[b]	5-HT$_3$[c]	5-HT$_4$[d]	5-HT$_5$[e]	5-HT$_6$[e]	5-HT$_7$[e]	5-HT Uptake[f]
5-MeODMT	0.21	0.063	6.38	0.93	N.D.	0.10	0.003	2.47
5-HOAMT	0.37	0.098	0.66	0.34	N.D.	3.56	0.12	0.10
5-MeOAMT	1.25	1.00	>10	1.00	>10	0.413	0.089	>10
R-DOI	2.86	N.D.	>10	>10	>10	0.70	N.D.	>10

N.D., not determined.
[a] Radioligand [^{125}I](−)-iodocyanopindolol, rat striatum.
[b] [^{3}H]5-CT, bovine striatum.
[c] [^{3}H]GR 65630 [3-(5-methyl-1*H*-imidazol-4-yl)-1-(1-methyl-1*H*-indol-3-yl)-1-propanone], NIE-115 neuroblastoma cells.
[d] [^{3}H]GR 113808 [1-methyl-1*H*-indole-3-carboxylic acid, [1-[2-[(methylsulfonyl)amino]methyl]-4-piperidinyl]methyl ester], guinea pig striatum.
[e] [^{3}H]Lysergic acid dehydrogenase, human cloned.
[f] [^{3}H]Citalopram, human platelets.

Binding at Other Receptors and Enzyme Inhibition. The affinity was determined at α_1- and α_2 -adrenergic receptors for the four compounds that showed a high response in the in vivo assay (Table 4). These compounds had only weak, micromolar level affinity for the adrenergic receptors. This level of affinity was well below that observed for the known ocular hypotensive agents apraclonidine and brimonidine, which have high affinity at the α_{2A} receptor and modest affinity at the α_{2C} receptor.

The monoamine oxidase A and B inhibitory activity of four of the compounds of particular interest was assessed, along with 5-fluoro-α-methyltryptamine, which is known to have a high affinity for MAO-A (Table 5). Neither 5-HOAMT, 5-MeOAMT, *R*-DOI, nor *S*-DOI showed significant inhibition of either MAO-A or MAO-B when tested at concentrations up to 10 μM, i.e., $K_i > 10$ μM. A significant inhibition of MAO-A activity was observed with 5-fluoro-α-methyltryptamine at concentrations of 100 nM and 10 μM, which is in agreement with the reported enzyme activity for this reference compound. (Tadano et al., 1995). No significant activity at MAO-B was observed for 5-fluoro-α-methyltryptamine, also in agreement with reported observations, $K_i > 10$ μM.

Animal Studies

Effect of Compounds on Intraocular Pressure in the Conscious Cynomolgus Monkey. Instillation of 5-HT into the lasered (hypertensive) eye of the monkeys resulted in a modest IOP reduction of 18% at the 3-h postdose reading; the pressure returned to the baseline value by 6 h (Fig. 1A). Topical ocular administration, in a similar manner, of the selective 5-HT$_{1A}$ agonists *R*-8-OH-DPAT (300 μg) and DP-5-CT (*N,N*-dipropyl-5-carboxamidotryptamine) (250 μg) did not lead to a decrease in IOP (Table 6). Similarly, compounds with 5-HT$_{1A}$ agonist/α_1-antagonist activity (Table 1), flesinoxan (250 μg), 5-methyl-urapidil (1000 μg), and WB-4101 (300 μg), did not lower IOP in the monkey (Table 6).

None of the 5-HT$_2$ antagonists that were evaluated lowered IOP in the conscious monkey following topical ocular application: ketanserin, M-100907, cinanserin (selective 5-HT$_{2A}$ antagonists), ritanserin (5-HT$_{2A/2C}$-selective), SB-206553 (5-HT$_{2B/2C}$-selective), RS-102221, and SB-242084 (5-HT$_{2C}$-selective) (Table 6).

The tryptamines, 5-methoxytryptamine (5-MeOT) and 5-hydroxy-*N*-methyltryptamine (5-HOMT), which showed potent agonist activity at both 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors (Table 1), displayed only a marginal transient decrease in IOP (<20%) following topical administration (Fig. 1). However, potent IOP reduction (>25%) was observed in the monkey following topical ocular administration of the dual 5-HT$_{1A}$/5-HT$_{2A}$ receptor agonists 5-MeODMT, 5-hydroxy-*N,N*-dimethyltryptamine (5-HODMT), 5-HOAMT, and 5-MeOAMT (Fig. 1).

Topical ocular administration of the selective 5-HT$_2$ agonist (±)-DOI (150 μg) resulted in a pronounced reduction in pressure (31%) relative to that observed for vehicle alone (Fig. 2A). Similarly, the higher affinity *R*-enantiomer of DOI decreased pressure in a dose-dependent manner, providing a maximum reduction of 34% at the 6-h postdose measurement following 100- and 300-μg doses. Evaluation of the *S*-enantiomer of DOI at comparable doses (100 and 300 μg) and in an identical manner did not result in a reduction of IOP in the monkey (Fig. 2B). Additionally, *R*-DOI (300 μg) was evaluated in the normal (nonlasered) left eye of the animals. A significant reduction of IOP was observed in the treated normal left eye with a peak reduction of 24% at the 3-h reading. No reduction of pressure was observed in the contralateral, un-

TABLE 4

Binding affinities (K_i; micromolar) for selected compounds at α adrenergic receptors

Values for α_{1A}-, α_{1B}-, and α_{2B}-receptors determined by NovaScreen Biosciences, using their standard protocols as described for Table 3. Values for α_{2A} and α_{2B} receptors were conducted at Alcon and are the mean of at least three experiments ± S.E.M. where appropriate.

Compound	α_{1A}[a]	α_{1B}[b]	α_{2A}[c] [IC$_{50}$]	α_{2B}[d]	α_{2C}[c] [IC$_{50}$]
5-MeODMT	>10	>10	13.5 ± 1.40	~1	6.93 ± 3.63
5-HOAMT	>10	>10	29.6 ± 3.62	>10	10.4 ± 3.36
5-MeOAMT	>10	>10	21.2 ± 12.3	>1	>10
R-DOI	>10	>10	>10	2.11	10.1 ± 0.60
Apraclonidine			0.033 ± 0.005		0.195 ± 0.069
Brimonidine			0.027 ± 0.005		0.434 ± 0.088

[a] Radioligand [^{3}H]7-MeO-prazosin, rat cerebral cortex.
[b] [^{3}H]7-MeO-prazosin, rat liver.
[c] [^{3}H]Clonidine, cloned human receptor.
[d] [^{3}H]MK-912 [(2*S*, 12b*S*)1',3'-dimethylspiro(1,3,4,5'6,6',7,12b-octahydro-2*H*-benzo[*b*]furo[2,3-*a*]quinolizine)-2,4'-pyrimidin-2'-one], NG108 neuroblastoma cells.

TABLE 5

Percentage inhibition of monoamine oxidase A and B activity in the presence of selected α-methyl-arylethylamines

Determinations were done by NovaScreen Biosciences; values are the mean of two experiments ± standard deviation.

Compound	Monoamine Oxidase A[a]			Monoamine Oxidase B[b]		
	1 nM	100 nM	10 μM	1 nM	100 nM	10 μM
5-HOAMT	12.1 ± 7.43	10.7 ± 6.63	21.2 ± 0.94	10.5 ± 6.56	6.08 ± 12.9	−15.4 ± 3.95
5-MeOAMT	0.25 ± 7.63	−9.63 ± 1.83	18.7 ± 13.2	−14.6 ± 1.99	−12.4 ± 0.52	−12.0 ± 4.85
R-DOI	7.63 ± 0.60	−1.59 ± 6.87	26.6 ± 8.82	−6.63 ± 2.71	−5.62 ± 3.09	2.59 ± 0.61
S-DOI	0.01 ± 25.6	9.09 ± 11.2	20.5 ± 8.30	−19.3 ± 8.89	−15.8 ± 7.47	−17.6 ± 1.76
5-FAMT	−2.14 ± 19.6	40.5 ± 13.5	98.8 ± 0.44	−10.4 ± 2.62	−4.44 ± 10.5	−7.29 ± 0.41

[a] Rat brain, [^{14}C]5-HT.
[b] Rat brain, [^{14}C]phenylethylamine.

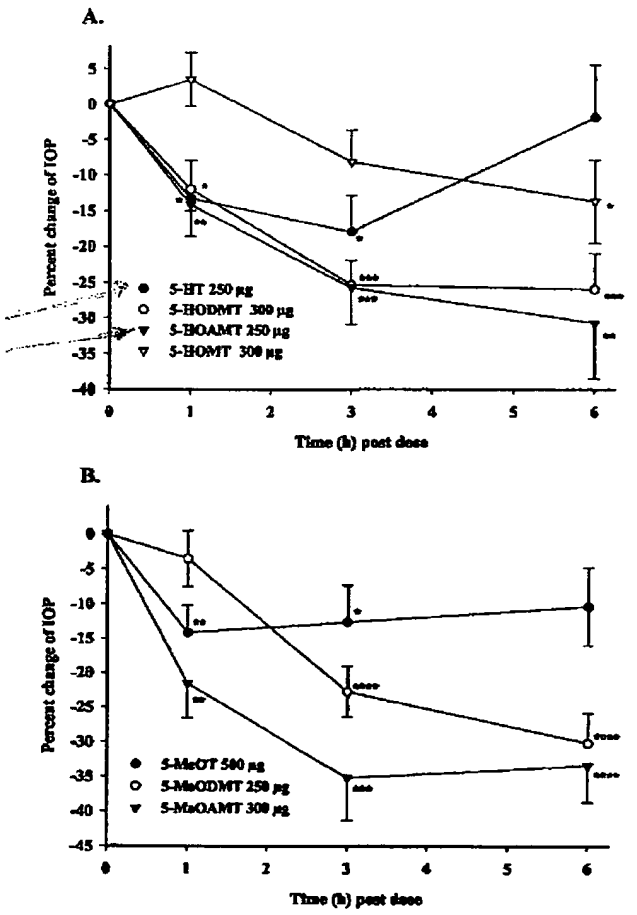


Fig. 1. IOP response to mixed 5-HT_{1A}-5-HT_2 agonists after topical ocular administration to the hypertensive eye of conscious cynomolgus monkeys. The values for each test compound are the mean of at least eight animals ± S.E.M. For clarity, the IOP response of the vehicle control (n = 5) for each experiment is not plotted. The maximum IOP reduction observed for any of the vehicle control groups did not exceed inherent model variability of −15%. A, 5-HT (acetate, pH 4.0), 5-HOMT and 5-HODMT (phosphate, pH 7.4), and 5-HOAMT (acetate, pH 5.0). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. B, 5-MeOT and 5-MeOAMT (phosphate, pH 7.4), and 5-MeODMT (phosphate, pH 6.2). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

treated laser-induced hypertensive eye (Table 7). No mydriasis or miosis was observed in either the treated or the contralateral eye during the course of the study.

Discussion

The results presented here show that none of the evaluated 5-HT_2 receptor antagonists lowered IOP in the conscious lasered monkey following topical ocular application. Ketanserin, a 5-HT_{2A}-selective antagonist that also has α_1 antagonist activity, has been reported to lower IOP in rabbit (Krootila et al., 1987) and humans (Mastropasqua et al., 1997) but was without efficacy in our conscious nonhuman primate model of ocular hypertension. A similar lack of efficacy was observed for other 5-HT_2 antagonists including M-100907 (5-HT_{2A}), SB-206553 (5-$HT_{2B/2C}$), RS-102221 (5-HT_{2C}), and SB-242084 (5-HT_{2C}) (Table 6). These observations demonstrate that antagonism of 5-HT_2 receptor subtypes does not lead to an IOP reduction in our monkey model.

None of the compounds tested that exhibited high affinity and high potency at the 5-HT_{1A} receptor, but lacked affinity for, or agonist activity at, the 5-HT_{2A} receptor [e.g., (*R*)-8-OH-DPAT, DP-5-CT, 5-Me-urapidil; Table 1] caused a decrease in IOP in the conscious monkey (Tables 1 and 6). Therefore, a 5-HT_{1A} agonist response alone is not sufficient to produce an IOP reduction in the primate model. Since the selective 5-HT_{1A} receptor agonist *R*-8-OH-DPAT has been shown to lower IOP in rabbits (Chu et al., 1999; Osborne et al., 2000), the lack of effect of this compound in the monkey also suggests a species difference with regard to the responsiveness to 5-HT_{1A} agonists. The mixed 5-HT_{1A} agonist/α_1 antagonists flesinoxan, 5-methyl-urapidil, and WB-4101 have been reported to lower IOP in rabbits (Osborne et al., 2000; Chidlow et al., 2001). The latter two compounds decreased IOP in sedated monkeys (Wang et al., 1997; Podos et al., 1999). However, these compounds did not lower IOP in our conscious monkey model (Table 6), indicating further that 5-HT_{1A} receptors are not directly involved in mediating the IOP-lowering response in our monkey model.

Several tryptamine ligands that have agonist activity at both the 5-HT_{1A} and 5-HT_2 receptors (Table 1) were also evaluated to assess their ability to affect IOP in our primate model: 5-HT, 5-MeOT, 5-HOMT, 5-HODMT, 5-MeODMT, 5-HOAMT, and 5-MeOAMT (Fig. 3). When dosed topically to the eye of the monkey, serotonin showed a weak but significant effect at the 1-h and 3-h time points; however, at the 6-h reading, the pressure had returned to baseline value (Fig. 1A). This relatively low efficacy of 5-HT for lowering IOP may be explained partially by the lack of its receptor selectivity. In addition, 5-HT is readily metabolized by deamination, so that the reversal by 6 h postdose probably reflects the metabolic instability of 5-HT.

Methylation of 5-HT either on the carbon α to the primary amine or on the amine itself has been shown to decrease

TABLE 6
IOP response induced by selective 5-HT$_{1A}$ receptor agonists and 5-HT$_2$ receptor antagonists after topical ocular administration to the hypertensive eye of conscious cynomolgus monkeys

Compound[a]	Reported Receptor Selectivity	Baseline IOP[b]	Percentage IOP Reduction 3 h Postdose[b]
		mm Hg	
(*R*)-8-OH-DPAT[c]	5-HT$_{1A}$ agonist[d]	30.4 (1.95)	4.0 (4.84)
DP-5-CT[c]	5-HT$_{1A}$ agonist[d]	38.0 (2.99)	11.9 (1.67)
Flesinoxan[e,g]	5-HT$_{1A}$ agonist α_1-antagonist[d]	37.4 (3.88)	11.6 (1.91)
5-Me-urapidil[h]	5-HT$_{1A}$ agonist α_1-antagonist[d]	45.0 (3.75)	16.8 (9.38)
WB-4101[c]	5-HT$_{1A}$ agonist α_1-antagonist[d]	45.2 (3.45)	9.6 (5.38)
Ketanserin[f]	5-HT$_{2A}$ antagonist α_1 antagonist	40.6 (3.53)	3.4 (6.39)
M-100907[c]	5-HT$_{2A}$ antagonist α_1 antagonist	35.8 (2.04)	15.8 (3.81)
Cinanserin[c]	5-HT$_2$ antagonist	40.9 (3.45)	6.8 (2.18)
Ritanserin[c]	5-HT$_2$ antagonist α_1 antagonist	34.6 (1.96)	10.2 (3.58)
SB-206553[c]	5-HT$_{2B/2C}$ antagonist	39.1 (2.32)	13.4 (4.41)
RS-102221[c]	5-HT$_{2C}$ antagonist	35.8 (3.17)	7.9 (2.62)
SB-242084[c]	5-HT$_{2C}$ antagonist	35.6 (2.20)	12.2 (1.69)

[a] Highest dose tested; vehicle, phosphate-buffered saline, pH 7.4.
[b] Values are the mean of at least eight animals ± S.E.M.
[c] Highest dose tested, 300 μg.
[d] See Table 1 for 5-HT$_{1A}$ receptor binding and functional data.
[e] Highest dose tested, 500 μg.
[f] Highest dose tested, 250 μg.
[g] pH 6.2.
[h] Highest dose tested, 1000 μg.

metabolic deamination; therefore, methylated 5-HT analogs were evaluated for their efficacy in reducing IOP. *N*-Methyl-5-HT, which is also readily deaminated, showed little effect on IOP. However, 5-HODMT evoked a greater reduction (26%) at the 3-h reading than that observed for 5-HT, and this reduction was maintained through the 6-h reading, suggesting a greater metabolic stability for 5-HODMT (Fig. 1A). The profile for the IOP reduction achieved with 5-HOAMT was similar to that observed for 5-HODMT; however, the magnitude of the pressure reduction (31%) at the 6-h time point was greater (Fig. 1A).

Evaluation of *O*-methylated derivatives of 5-HT, 5-HODMT, and 5-HOAMT provided pressure reduction profiles that were qualitatively comparable to those of the corresponding hydroxyl compounds (Fig. 1B). Therefore, as anticipated, the tryptamines having the greater metabolic stability were very effective in lowering pressure in the conscious ocular hypertensive cynomolgus monkey. To our knowledge, none of these compounds have been previously evaluated for their IOP response in either normotensive animals or animal models of ocular hypertension.

To further assess the involvement of 5-HT$_2$ receptor activation in the reduction of IOP, selective 5-HT$_2$ agonists were evaluated. The prototypic selective 5-HT$_2$ agonist, (±)-DOI, is a high-affinity partial agonist with modest functional selectivity for the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors relative to the 5-HT$_{2C}$ receptor at cloned human or cloned rat receptors (Porter et al., 1999; Vickers et al., 2001). We confirmed the high-affinity and high-agonist potency of (±)-DOI and its enantiomers at the 5-HT$_{2A}$ receptor and also the inactivity of these compounds at the 5-HT$_{1A}$ receptor (Table 1). When tested in the lasered ocular hypertensive monkey, (±)-DOI (150 μg) demonstrated a pronounced 31% reduction of IOP at the 6-h time point (Fig. 2A). In view of the favorable response observed with (±)-DOI, the individual enantiomers of this compound were evaluated to assess their effect on IOP. The more potent *R*-enantiomer (*R*-DOI) was efficacious in decreasing IOP and did so in a dose-dependent manner; *S*-DOI did not lower IOP (Fig. 2B). The favorable IOP response to *R*-DOI observed in the monkey demonstrates that agonist activity at the 5-HT$_2$ receptor is sufficient to lower IOP in this model of ocular hypertension.

The complete lack of response observed for *S*-DOI in the monkey was unanticipated in view of its relatively high affinity and potency at the 5-HT$_{2A}$ receptor compared with other compounds that did lower IOP (Table 1). This lack of response suggests that *S*-DOI lacks the requisite efficacy at the 5-HT$_2$ receptor or is selectively metabolized. A stereoselective pharmacologic response has been reported for some 1-phenyl-2-aminopropane enantiomeric pairs in other in vivo studies (Shulgin, 1973; Aldous et al., 1974; Shulgin and Shulgin, 1991). This effect has been suggested to arise from the selective metabolism of the less active enantiomer. Indeed, both in vitro and in vivo metabolism studies with 1-phenyl-2-aminopropanes, such as 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane and amphetamine, have demonstrated the occurrence of stereoselective metabolism (Weinkam et al., 1976; Gunaratna and Kissinger, 1998). An evaluation of the metabolism of DOI and its enantiomers in ocular tissues is therefore warranted in view of the dramatically different IOP reduction responses observed for the enantiomers of this compound.

The mechanism through which 5-HT$_2$ agonists exert their IOP-lowering effect has not been established. It is, however, of interest to note that tryptamine analogs, and in particular, α-methyl-tryptamines, have been reported to inhibit MAO (Tipton et al., 1982; Tadano et al., 1995). MAO inhibitors have been reported to be effective in decreasing IOP in rabbit and cat (Colasanti and Trotter, 1982) and humans (Mehra et al., 1974). Also, MAO-A inhibition has been reported to potentiate the IOP-lowering effects of epinephrine (Maeda et al., 1988). To assess whether inhibition of MAO might be involved in the reduction of IOP observed for the α-methyl-

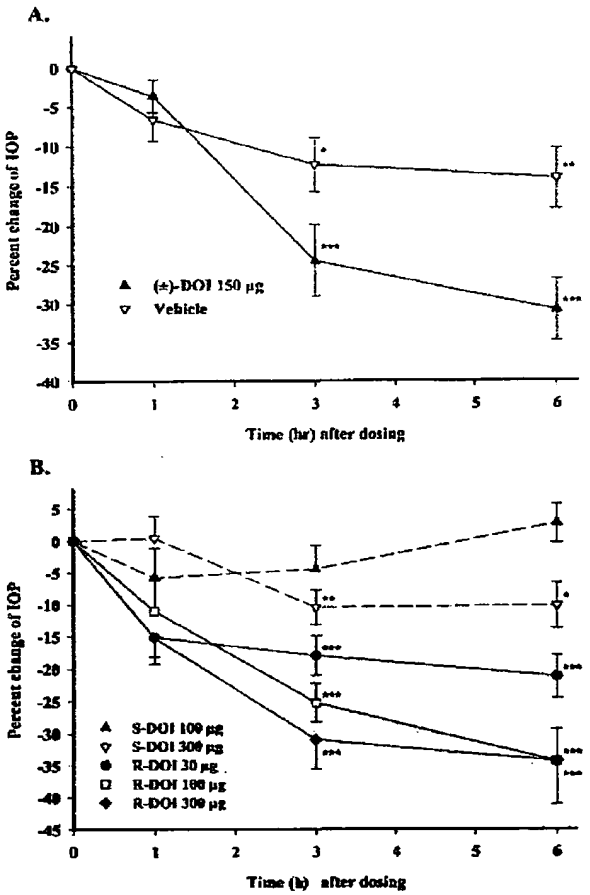


Fig. 2. IOP response after topical ocular administration of DOI to the hypertensive eye of conscious cynomolgus monkeys. The values for each test compound are the mean of at least eight animals ± S.E.M. A, (±)-DOI (phosphate, pH 7.4). Values for the vehicle are the mean of at least five animals ± S.E.M. *, $p < 0.02$; **, $p < 0.01$; ***, $p < 0.001$. B, Dose response of *R*-DOI and *S*-DOI (phosphate, pH 7.4). For clarity the IOP response of the vehicle control ($n = 6$) for each experiment is not plotted. The maximum IOP reduction observed for any of the vehicle control groups did not exceed inherent model variability of −15%. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

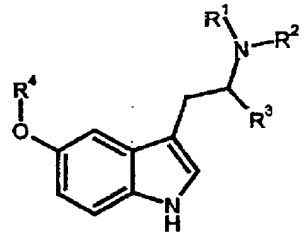
TABLE 7

IOP response induced by *R*-DOI after topical ocular administration to the normal eye of conscious cynomolgus monkeys

Dose[a]	Baseline IOP[b]	Percentage IOP Reduction (hours postdose)[b]		
		1	3	6
	mm Hg			
Untreated, OD[c]	34.9 (1.93)	5.0 (5.93)	11.1 (4.92)	10.9[d] (4.03)
100 µg, OS[e]	21.0 (1.11)	14.7[d] (4.65)	24.1[f] (4.84)	14.8[d] (6.05)

[a] Vehicle, phosphate-buffered saline, pH 7.4.
[b] Values are the mean of at least eight animals ± S.E.M.
[c] Lasered (hypertensive) eye contralateral to *R*-DOI treatment.
[d] $p < 0.05$.
[e] Normotensive eye.
[f] $p < 0.001$.

arylethylamines of the present study, the activity of selected compounds for rat brain MAO-A and MAO-B was determined. None of the compounds evaluated showed significant inhibition of MAO-A or MAO-B (Table 5). Therefore, it appears that inhibition of MAO is not involved in the ocular hypotensive response observed for 5-HOAMT, 5-MeOAMT, and *R*-DOI.



	R^1	R^2	R^3	R^4
5-HT	H	H	H	H
5-MeOT	H	H	H	Me
5-HOMT	Me	H	H	H
5-HODMT	Me	Me	H	H
5-MeODMT	Me	Me	H	Me
5-HOAMT	H	H	Me	H
5-MeOAMT	H	H	Me	Me

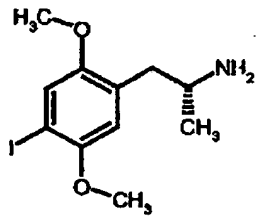


Fig. 3. Structures of substituted 5-hydroxytryptamines and *R*-DOI.

5-MeODMT (Weil and Davis, 1994), 5-MeOAMT (Kantor et al., 1980), and *R*-DOI (Glennon et al., 1982) have been shown to enter the central nervous system following systemic dosing, suggesting that a centrally mediated mechanism may be responsible for the reduction in IOP observed with 5-HT_2 agonists. However, the nonselective peripheral 5-HT_2 agonists 5-HOAMT (Baudrie and Chaouloff, 1992; Okada et al., 1995) and 5-HODMT (McBride, 2000) also decreased IOP in the monkey following topical administration. Furthermore, the topical application of *R*-DOI to the normal (nonlasered normotensive) eye of the monkeys resulted in a significant reduction of IOP in this treated normal left eye; however, no reduction of IOP was observed in the untreated (undosed) hypertensive contralateral eye (Table 7). Taken together, these observations suggest that a local ocular site of action appears to be sufficient for achieving the decrease in pressure observed for the 5-HT_2 receptor agonists evaluated here.

In summary, it has been demonstrated that 5-HT_2 receptors are involved in the control of intraocular pressure in the conscious lasered cynomolgus monkey. Agonists at these receptors have been identified as effective ocular

hypotensive agents in this primate model of ocular hypertension. Compounds that function as efficient agonists of the 5-HT$_2$ receptors should therefore be considered as potential agents for the control of intraocular pressure in the treatment of ocular hypertension and glaucoma in humans.

Acknowledgments

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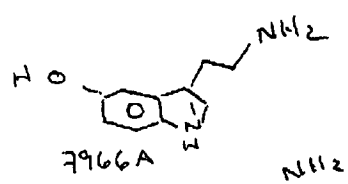
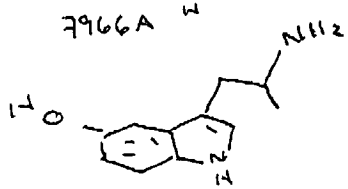
EXHIBIT 2

Physicochemical Data and Monkey IOP Response Data

Compound	% Sol 5.0	% Sol 7.4	D.C. 5.0	D.C. 7.4	pK	Receptor	Y, max %	MIOP dose, ug	Percent Decrease of IOP 1 hr	3 hr	6 hr	Comments	pK (lit)	logP (lit)
AL07966A serotonin	> 1	> 1	0	0.000159		endogenous	18	250 [pH 4.0]	13	18	2		4.9; 9.8; 11.1	
AL12170A aMe-5HT	0.678	0.647	0	0.015	none 3-10	2 ag	31	250 [pH 5.0, Ac]	14	26	31		10.3 (5MeO)	
AL12170A aMe-5HT						2 ag	30	150 [pH 5.0, Ac]	15	30	26			



7966A "5-HT"



12170A "5HO-AMT"